

20(S)-camptothecin (CPT)

10,11-methylenedioxy-20(S)-camptothecin (MDC): 10,11 = -O-CH<sub>2</sub>-O-

Figure 1: Structure of camptothecin (CPT) and methylenedioxycamptothecin (MDC)

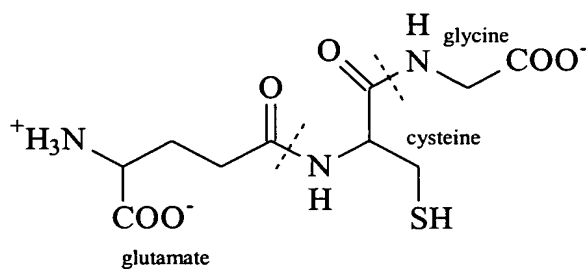


Figure 2: Structure of the tripeptide glutathione (GSH)

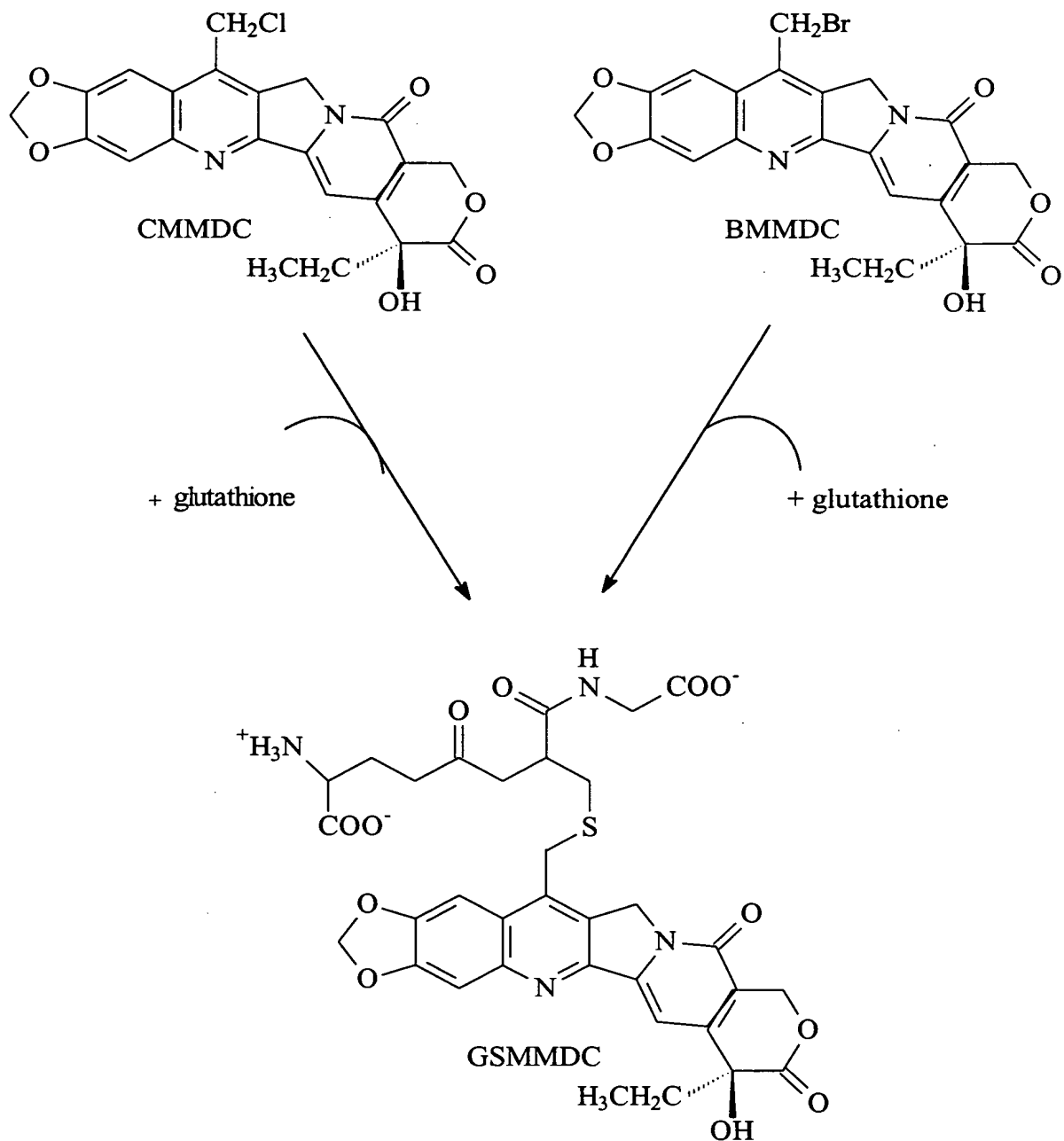
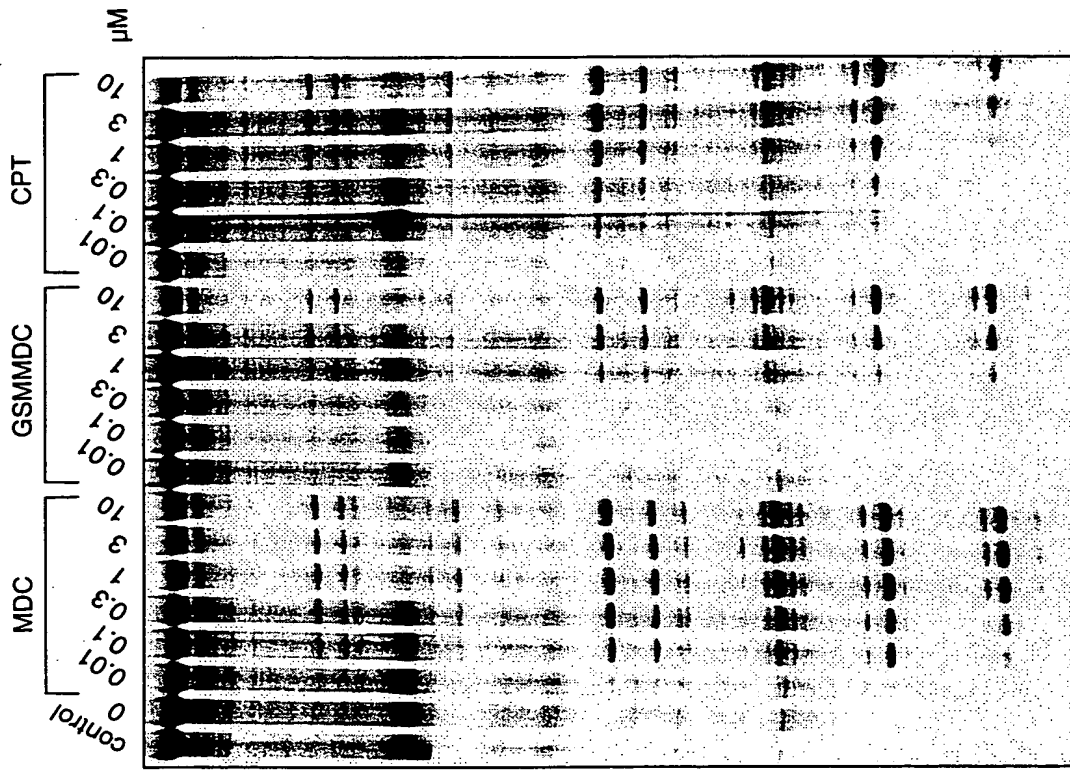


Figure 3. Reaction of 7-chloromethyl-10,11-methylenedioxy-20(S)-camptothecin (CMMDC) or 7-bromomethyl-10,11-methylenedioxy-20(S)-camptothecin (BMMDC) with glutathione yields the conjugate 7-(methyl-S-glutathionyl)-10,11-methylenedioxycamptothecin (GSMDC).

DNA cleavage produced by human top1 in pSK DNA  
 (GSMMDc = 7-chloromethyl-10,11-methylenedi xycamptothecin;  
 MDC = 10,11-methylenedioxycamptothecin; CPT = camptothecin)

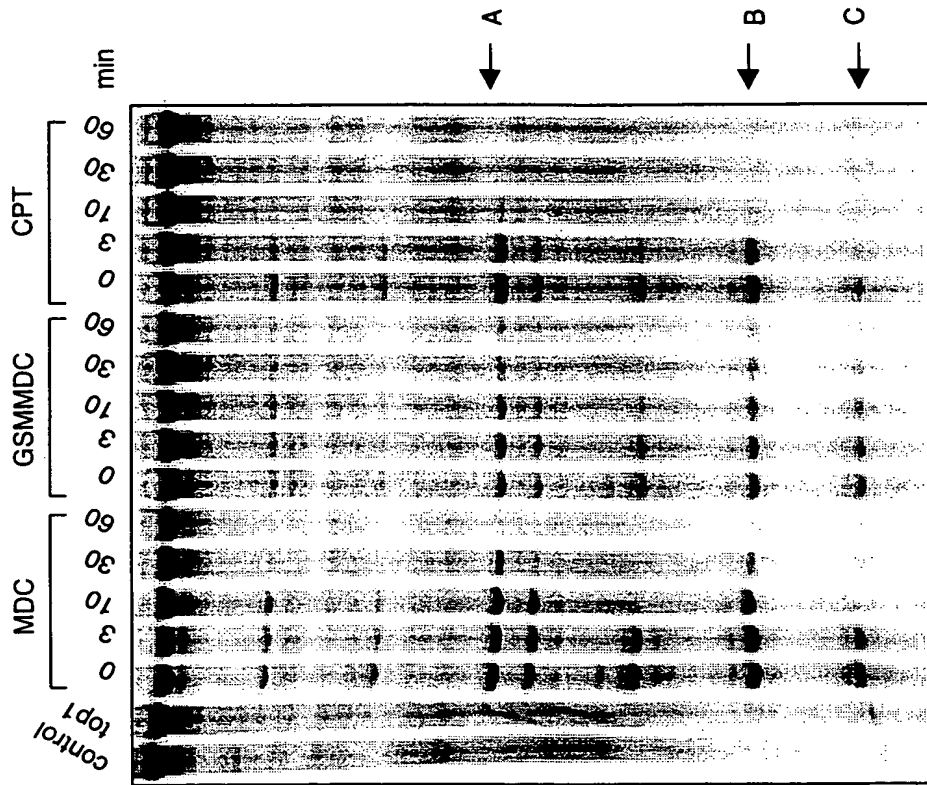
Figure 4:



30 minutes reactions  
 At room temperature;  
 Stopped with 0.5% SDS.  
 Sequencing gel

Reversal Kinetics of DNA cleavage produced by human topoisomerase I (GSMMD) = 7-chloromethyl-10,11-methylene-10,11-methylenedioxycamptothecin; MDC = 10,11-methylenedioxycamptothecin; CPT = camptothecin

Figure 5:



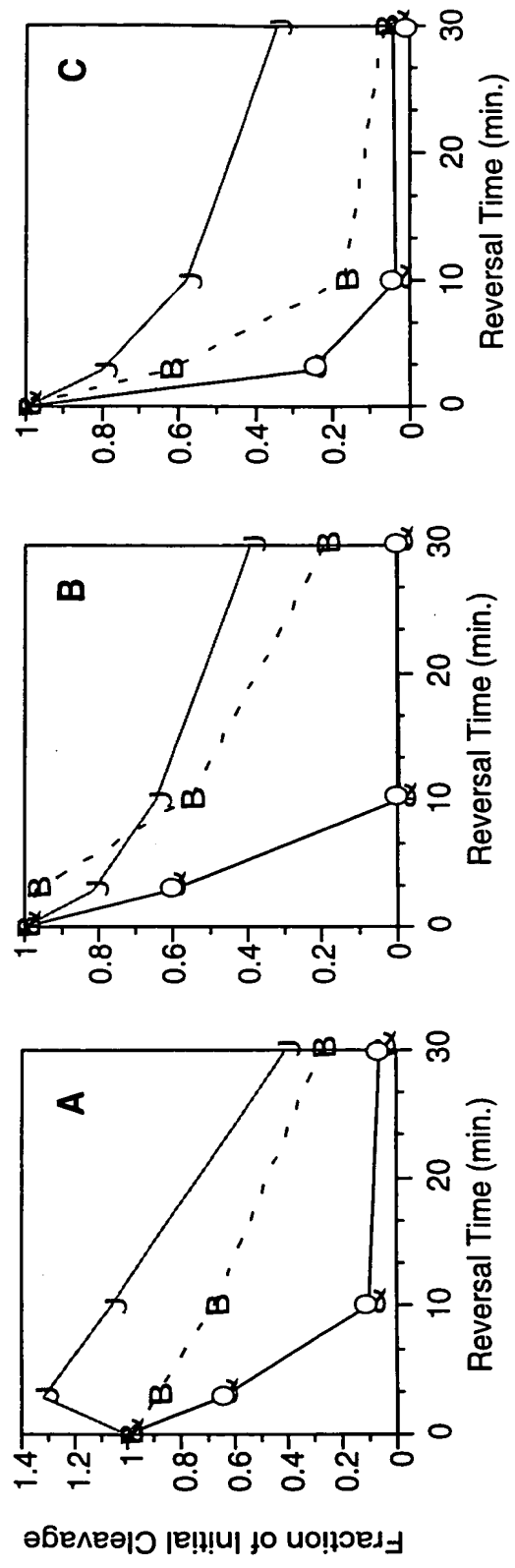
Reactions for 15 min at room temperature  
 Followed by addition of 0.35 M NaCl (final concentration) for the indicated times.  
 Reactions stopped with 0.5% SDS; Sequencing gel

Next figure shows the quantitation after PhosphorImager analysis

Reversal Kinetics of DNA cleavage produced by human topoisomerase I in pSK DNA  
(GSMMD = 7-chloromethyl-10,11-methylene-10,11-dioxycamptothecin;  
MDC = 10,11-methylene-10,11-dioxycamptothecin; CPT = camptothecin)

Figure 6:

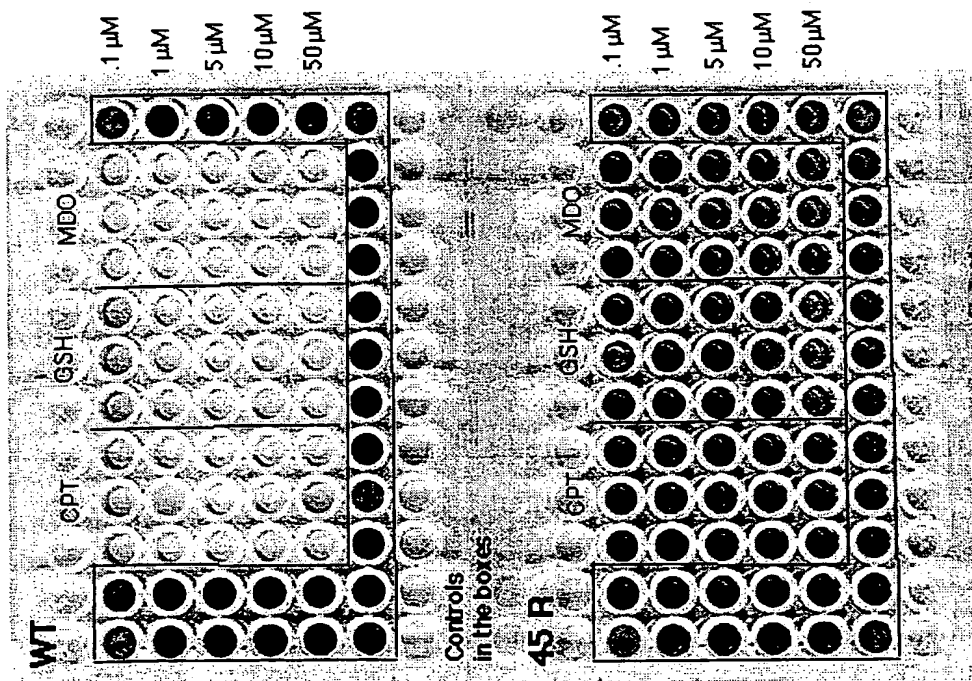
Quantitation by PhosphorImager of previous gel



J GSMMD CPT      B MDC      O CPT

MTT cytotoxicity assay on P388 WT and P388 45R cells treated continuously for 3 days with CPT derivatives. 022800 GK

Figure 7:



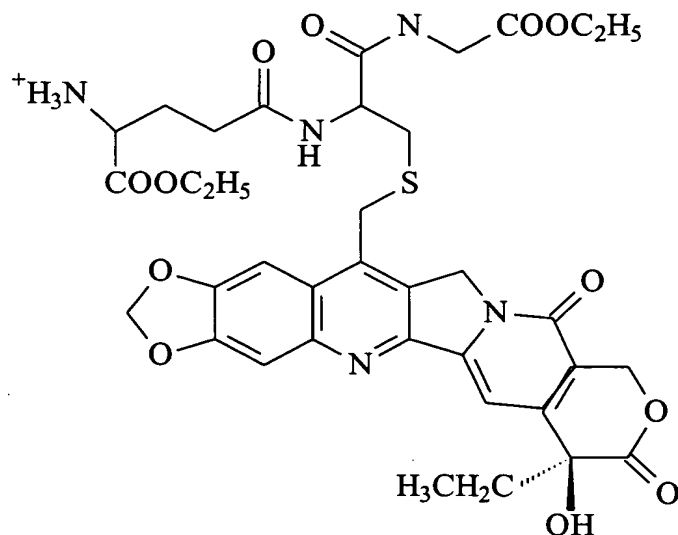


Figure 8: Diethylester of GSMMDc

$R_1 = \text{CH}_3\text{-CO-}; R_2 = \text{-OH}$

$R_1 = \text{H}; R_2 = \text{glycine}$

$R_1 = \text{amino acid}; R_2 = \text{amino acid}$

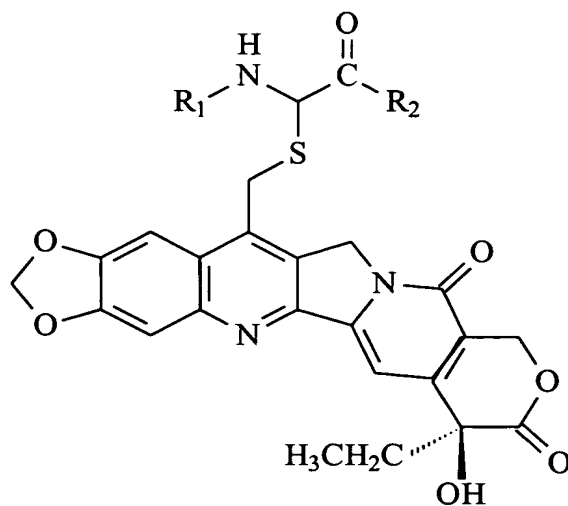


Figure 9: Camptothecin derivatives based on cysteinyl-conjugate